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Treatment with a peroxisomal proliferator activated receptor gamma agonist has a modest effect in the allergen challenge model in asthma: A randomised controlled trial

Duncan B. Richards [a,*], Philippe Bareille [a], Emma L. Lindo [b], Dean Quinn [c], Stuart N. Farrow [a]

[a] *Drug Discovery, GlaxoSmithKline, Stevenage CB2 2GG, UK*
[b] *Discovery Biometrics, GlaxoSmithKline, Greenford, UK*
[c] *P3 Research, Bowen Hospital, Wellington, New Zealand*

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Summary
Purpose: A considerable body of non clinical evidence has accumulated to support peroxisomal proliferator-activated receptor gamma agonists as candidate anti-inflammatory drugs in asthma. We utilized rosiglitazone as a tool compound in the inhaled allergen challenge model of asthma.
Methods: A single centre, double-blind, randomised, placebo controlled, two period cross-over study. Subjects received rosiglitazone 4 mg and placebo twice daily for 28 days in random order. On day 28, inhaled allergen challenge was performed 1 hour post-dose. A methacholine challenge was performed on day 29 and an adenosine monophosphate challenge on day 14. Exhaled nitric oxide was measured on days 1, 14, 28, 29. Blood was collected pre dose on days 1, 14 and 28 and analysed for markers associated with PPAR activity and systemic markers of inflammation.
Results: The late asthmatic reaction (LAR) change from post saline FEV_1 from 4–10 hrs post allergen on day 28 was statistically significant for the weighted mean LAR. The difference in weighted mean was 0.06 L (95% CI 0.01 to 0.11) which equates to a 15% attenuation of the response during placebo treatment. This was accompanied by trends in other markers of efficacy and anti-inflammatory activity but none were considered major effects.
Discussion: Treatment with a PPARγ agonist (rosiglitazone) was associated with a modest (15%) reduction in the late asthmatic reaction in the allergen challenge model of asthma. Based on

* Corresponding author. Tel.: +44 1223 296159.
E-mail addresses: duncan.b.richards@gsk.com (D.B. Richards), philippe.j.bareille@GSK.com (P. Bareille), emma.l.lindo@GSK.com (E.L. Lindo), dean@p3research.co.nz (D. Quinn), stuart.n.farrow@gsk.com (S.N. Farrow).

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the results of this study, PPARγ agonist monotherapy is unlikely to represent a clinically useful intervention in human asthma.

Registered with www.clinicaltrials.gov (NCT00318630).

Background

Inhaled glucocorticoids are highly effective and form the mainstay of asthma preventive therapy. Nevertheless they are not universally effective and may be associated with adverse systemic effects, especially at high doses. There remains substantial unmet need for effective alternative anti-inflammatory treatments in asthma. The peroxisome proliferator-activated receptors (PPARs) comprise three closely related members, PPARα, PPARδ and PPARγ. Synthetic ligands for PPARγ such as rosiglitazone (a thiazolidinedione) have benefits in diabetes mellitus via transcriptional regulation of lipid and glucose metabolism.[1,2] PPARγ receptors are expressed in a several important immune cells, which has prompted investigation of their potential as immune regulators and anti-inflammatories.

The PPARγ receptor has been shown to be up-regulated in asthmatic epithelium,[3] and PPARγ agonists in vitro have a range of effects on inflammatory cells including macrophage function, inhibition of tumour necrosis factor production, inhibition of T-cell proliferation and cytokine production. These and other observations have prompted investigation of PPARγ agonist effects in various models of allergic asthma and airway inflammation. Positive results in these studies have led to suggestions that PPARγ agonists might have utility for the treatment of human allergic asthma and chronic obstructive airways disease.[4] Most recently a study in smoking asthmatics showed an improvement in FEV_1 after 4 weeks rosiglitazone treatment compared with beclometasone treatment.[5]

Rosiglitazone (GSK) is licensed for the treatment of type II diabetes mellitus and we utilized it as a tool compound to explore the potential for an anti-inflammatory effect in mild asthmatics using the well-established inhaled allergen challenge model.

We performed a randomised double blind, placebo controlled, 2 period crossover study in steroid naïve asthma patients comparing the effects of rosiglitazone and placebo on airway hyper-reactivity and markers of inflammation.

Methods

Subjects

Thirty four steroid naïve patients with physician diagnosed asthma for at least 6 months were recruited. Subjects were required to be aged 18 to 55 years and non-smokers for at least 6 months with less than a 10 pack year history. At screening patients were required to have an $FEV_1 > 70\%$ predicted, have a positive skin test to either house dust mite, grass pollen or cat allergen, and to demonstrate both an early and late asthmatic reaction to one of these allergens when inhaled. The early asthmatic response (EAR) included a fall of $\geq$20% from the post saline value on at least one occasion between 10 and 30 minutes after the final concentration of allergen. The late asthmatic response (LAR) included a fall of $\geq$15% from the post saline value on at least three occasions, two of which were consecutive, between 4 and 10 h after the final concentration of allergen. They were also required to have an adenosine monophosphate (AMP) and methacholine PC_{20} (provocative concentration causing a 20% fall in FEV_1) less than 100 mg/ml and 8 mg/ml (or $PD20 \leq 3.2$ mg) respectively. All patients provided written informed consent. The study was approved by the local research ethics committee.

Study design

This was a single centre, double-blind, randomised, placebo controlled, two period cross-over study. Fig. 1 shows the study design. Eligible subjects received rosiglitazone 4 mg and matched placebo twice daily for 28 days in random order. During each treatment period subjects were instructed to take the study medication at the same time of day. The washout period was 14—28 days between treatment periods to allow sufficient time between allergen challenges and flexibility of study visit scheduling. Subjects had observed dosing on days 1, 14, 28 and 29 and were required to refrain from short-acting beta agonist use for 8 hrs prior to each clinic visit. Heart rate, blood pressure and forced expiratory volume in 1 second (FEV_1), were measured on days 1, 14, 28, and 29. Pedal oedema was measured on days 1, 14, and 28. On day 14, subjects underwent an AMP challenge within 1—3 hours post-dose. On day 28, an inhaled allergen challenge was performed 1 hour post-dose. A methacholine challenge was then performed on day 29 at 23 hours post allergen challenge (24 hours post dose). Details of the bronchial challenges are provided in the online data supplement. Exhaled nitric oxide (FE_{NO}) was measured using the NIOX analyser at a flow of 50 mL/s. Measurements were taken pre dose on

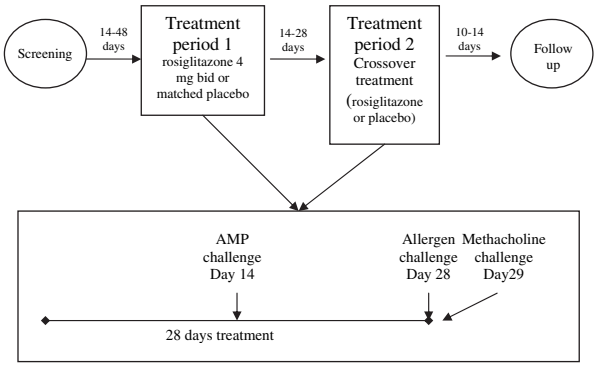

Figure 1 Study design.

days 1, 14, 28, pre challenge on day 29, and post challenge on days 14 and 29. Blood was collected pre dose for biomarker analysis on days 1, 14 and 28; it was analysed for markers of PPAR-γ activity (specifically adiponectin) and for systemic markers of inflammation. Sputum was collected pre dose on Day 1, and post challenge and after exhaled nitric oxide measurement on Days 14 and 29. Adverse events and beta agonist use were monitored throughout the study with the aid of diary cards.

Statistical analysis

Bronchial challenge endpoints were analysed using mixed effect models with treatment and period fitted as fixed effects and subject fitted as a random effect. Subject's overall mean baseline and period baseline were fitted as covariates (fixed effects). Confidence intervals (CI) where quoted are two-sided 95% CIs. Statistical significance at the 5% level is concluded where the 95% confidence interval for the treatment difference excludes 0 for the challenge endpoints and excludes 1 for the exhaled NO endpoints. Further details regarding the statistical methods are described in the online data supplement.

Results

Thirty four subjects (see Table 1 for demography) were planned and randomised of whom thirty two completed the study. One subject was withdrawn following Period 1 (rosiglitazone) on Day 50 due to a protocol violation (positive drugs of abuse test). One subject was withdrawn prior to completing Period 1 (rosiglitazone) on Day 10 due to an adverse event of tachycardia associated with a feeling of nervousness. The numbers of patients screened, excluded, randomised and withdrawn are summarised in Fig. 2.

Study medications were well tolerated. The most frequently reported adverse event was headache (reported by 12 subjects (38%) on placebo, 14 (41%) on rosiglitazone). Five adverse events (AEs) were rated as severe intensity; no events fulfilling the criteria for a Serious Adverse Event were reported. Following rosiglitazone, the AEs judged to be of severe intensity were one episode of sinusitis, two episodes of muscle spasm, and one episode of influenza. Following placebo, one subject reported a severe AE of sinusitis, which resolved and was judged to be unrelated to treatment. This subject had also reported severe sinusitis

Table 1 Subject demography.

Age, yr	30.5 (20—46)
Duration of asthma, yrs	21.94 (8—39)
Sex	21 male/13 female
FEV_1, L	3.45 (1.93—5.69)
FEV_1, % predicted	89.3 (71.0—123.4)
FE_{NO}, ppb	69.3 (16.1—216.1)
Allergens used for bronchial challenge	20 dust mite, 7 cat hair, 5 grass mix

Definition of abbreviations: FE_{NO} = exhaled breath nitric oxide. Data were collected before the first treatment period. Values represent mean (range), except for FE_{NO} data, which are presented as geometric mean (range).

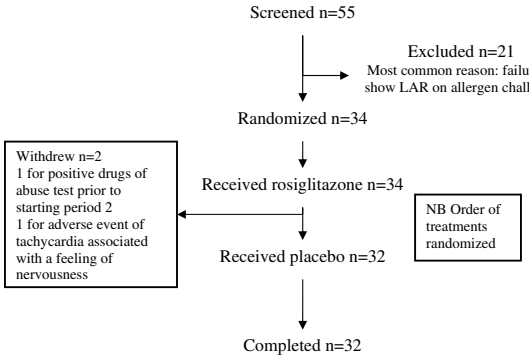

Figure 2 Flow of patients through study, showing number screened, randomised, withdrawn, and completed. Excluded patients did not fulfil the inclusion criteria (majority did not demonstrate a late asthmatic reaction).

following rosiglitazone. No treatment related trends were observed in safety laboratory parameters, blood pressure, ECG parameters, beta agonist reliever use and pre-dose FEV_1 values. Most pedal oedema ratings were grade 0 (<1 mm) throughout the study. One subject had an oedema rating of grade 2 (3 to 5 mm) at Day 28 on placebo and one subject had an oedema rating of grade 2 at Day 28 on rosiglitazone. None of the cases of peripheral oedema were considered clinically significant.

Allergen challenge

Early asthmatic response (EAR)

The EAR FEV_1 change from post saline baseline on day 28 was similar during treatment with roglitazone and placebo (Fig. 3). There was no significant difference for either the minimum FEV_1 or weighted mean FEV_1 change from baseline endpoints (Table 2).

Late asthmatic response (LAR)

The change from post saline baseline FEV_1 from 4—10 hrs post allergen on day 28 was not statistically significant for the minimum LAR during treatment with rosiglitazone compared with placebo but was statistically significant for the weighted mean LAR (Fig. 3; Table 2). The difference in weighted mean was 0.06 L (95% CI 0.01 to 0.11) which equates to a 15% attenuation of the response during placebo treatment.

Methacholine challenge

The adjusted geometric mean methacholine PC20 (mg/ml) performed 23 hours after the allergen challenge were 0.06 (95% CI 0.05 to 0.08) and 0.07 (95% CI 0.05 to 0.09) after treatment with rosiglitazone and placebo respectively. There was no statistically significant difference between the methacholine reactivity during dosing with rosiglitazone and placebo (mean doubling dose difference −0.20, 95% CI −0.62 to 0.22).

AMP challenge

During the course of the study a problem emerged with the supply of AMP challenge agent that made it impossible to

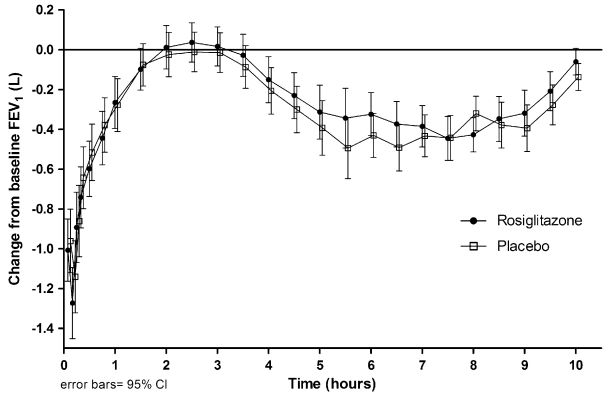

Figure 3 Early and late asthmatic response to inhaled allergen challenge after 28 days of treatment with either rosiglitazone or placebo. Adjusted means and 95% confidence intervals of change in FEV_1 compared with post-saline value are shown.

continue with this part of the investigation. AMP challenge data were therefore missing for 12 subjects in each of the treatment groups owing to this supply problem.

The adjusted geometric mean AMP PC20 (mg/mL) 1–3hrs post-dose on day 14 were 1.01 (95% CI 0.55 to 1.89) and 1.49 (95% CI 0.79 to 2.80) after treatment with rosiglitazone and placebo respectively. There was no statistically significant difference between the AMP reactivity during dosing with rosiglitazone and placebo (mean doubling dose difference −0.55, 95% CI −1.59 to 0.48).

Exhaled NO

Geometric mean and 95% confidence intervals for pre-dose FE_{NO} concentrations on days 1, 14, and 28 are shown in Table 3. Measurements were also made pre-challenge on day 29 (24 hours post allergen challenge) and after the challenges on days 14 (AMP) and 29 (methacholine); these data are summarised in the supplement (online data supplement Table E1). FE_{NO} (pre dose) in the rosiglitazone group was reduced on average by 16% from baseline on Day 14 and by 24% on Day 28. In the placebo group, FE_{NO} was reduced on average by 5% from baseline on Day 14 and by 12% on Day 28. There was statistical evidence of a difference in pre-dose FE_{NO} between the rosiglitazone and placebo treatment groups on days 14 and 28. On average, pre-dose FE_{NO} was reduced by 12% (95% CI 1.3 to 22% reduction) on Day 14 and 14% (95% CI 1.5 to 25% reduction) on Day 28 following administration of rosiglitazone compared with placebo.

Biomarkers

Adiponectin was measured as a pharmacodynamic measure of PPARγ activity (and hence also of compliance). There was a substantial increase in the average blood concentration of adiponectin (7.1 μg/L on Day 1 to 17.2 μg/L on Day 28), as expected after treatment with rosiglitazone. No elevation of adiponectin was observed during treatment with placebo (Day 1 7.5 μg/L; Day 28 6.5 μg/L). There were no major effects on hsC-reactive protein, interferon (IFN)-γ, interleukin (IL)-4, IL-6, IL-13, matrix metalloproteinase (MMP)-9 or tumour necrosis factor (TNF)-α, although these markers tended to be decreased after rosiglitazone (online data supplement Table E2). There were no trends observed in the blood differential white cell count measured on days 1, 14, and 28 (online data supplement Table E3).

Sputum differential counts were not analysed and are not presented owing to the low proportion of samples that were assessed as viable.

Discussion

PPARγ is expressed widely in the lung, in inflammatory, resident, and structural cells.[6] There is a considerable body of *in vitro* findings suggesting that PPARγ agonists have anti-inflammatory effects. For example, they reduce TNF, IL1 and IL6 production in airway epithelial cells, they reduce eosinophil migration and killing, and reduce IL2, IL5 and IFNγ production by T cells.[7–9]

The *in vitro* work has been supplemented by work in the mouse ovalbumin model of airway hyper-responsiveness. Treatment with a PPARγ agonist (ciglitazone) during sensitization, or immediately prior to allergen challenge markedly reduced airway hyper-responsiveness and inflammatory infiltrate into the lungs.[10,11] The doses of ciglitazone administered are similar to those required in animal models of diabetes.

In man there is evidence that PPARγ is upregulated in the airway epithelium and smooth muscle cells of those with asthma.[3] Treatment with corticosteroids reduces

Table 2 Early and Late asthmatic FEV_1 responses.

Absolute Change from Baseline Endpoint (L)	Adjusted Mean (95% CI)		Treatment Difference (95% CI[a]) Rosiglitazone - Placebo	% Attenuation from Placebo Response
	Rosiglitazone (N = 34)	Placebo (N = 32)		
Early Asthmatic Response (EAR)				
Minimum	−1.37 (−1.54, −1.19)	−1.30 (−1.48, −1.12)	−0.07 (−0.28, 0.15)	−5
Weighted Mean	−0.37 (−0.48, −0.26)	−0.34 (−0.45, −0.23)	−0.03 (−0.15, 0.09)	−9
Late Asthmatic Response (LAR)				
Minimum	−0.84 (−0.93, −0.74)	−0.90 (−1.00, −0.80)	0.06 (−0.03, 0.16)	7
Weighted Mean	−0.32 (−0.37, −0.27)	−0.38 (−0.43, −0.32)	0.06 (0.01, 0.11)	15

[a] A 95% CI for the treatment difference excluding 0 indicates statistical significance at the 5% level.

Table 3 Summary of exhaled nitric oxide data (FE_{NO} ppb).

Day	Time	Placebo ($N = 32$)		Rosiglitazone ($N = 34$)	
		n	Geometric Mean (95% Confidence Interval)	*n*	Geometric Mean (95% Confidence Interval)
1	Pre-dose	32	68.23 (54.48, 85.41)	34	74.09 (59.07, 92.92)
14	Pre-dose	30	66.05 (51.18, 85.23)	32	60.34 (47.72, 76.29)
28	Pre-dose	32	64.18 (50.02, 82.33)	33	53.54 (42.62, 67.24)

expression of PPARγ, but this observation does not elucidate whether this is because PPARγ contributes to the pathophysiology or is simply a marker of inflammatory process. There are 2 case reports of patients with asthma reporting reduced wheeze when given a PPARγ agonist (pioglitazone) for the treatment of type II diabetes.[12]

Taken together these data suggest that PPARγ agonists may have anti-inflammatory effects that are of clinical benefit in asthma, but evidence in humans is lacking. This study used rosiglitazone as tool compound to explore this further in humans.

Rosiglitazone is a high affinity ($IC_{50} = 10$ nM) PPARγ agonist.[13] It also demonstrates considerable selectivity for PPARγ over other PPAR subtypes (α and δ).[14]

The inhaled allergen challenge model is a well established one in which the LAR (4-10 hours post allergen) appears to correlate with the extent of airway inflammation and disease activity. Recruitment of activated eosinophils, key cells in the pathogenesis of asthma, underlies the late bronchoconstrictor response to allergen challenge.[15] The inflammatory response initiated by allergen challenge extends beyond the changes in FEV_1 seen in the early and late asthmatic response, and can be observed as increased bronchial hyper-responsiveness for several days after allergen challenge.[16] Inhaled corticosteroids (including budesonide and fluticasone) reduce the airway hyper-responsiveness seen after allergen challenge compared to placebo. The inclusion of a methacholine assessment on Day 29 increased the sensitivity of the study to detect an anti-inflammatory effect of treatment with a PPARγ agonist.

AMP is an indirect stimulus of bronchoconstriction, although its precise mechanism of action is unknown. It is best validated in terms of the action of corticosteroids.[17,18] This challenge was included on day 14 as a secondary endpoint because studies of inflammatory biomarkers have shown a statistically significant effect of treatment with rosiglitazone by Day 14 (the effect is maximal by Day 28 when the allergen challenge (primary endpoint) was assessed).[19]

Treatment with PPARγ agonists is associated with a rise in plasma adiponectin and this is a useful pharmacodynamic marker. Treatment with rosiglitazone 4 mg bid for 28 days in this study was associated with a substantial rise in plasma adiponectin. The magnitude of the change was similar to that which has previously observed in patients with type II diabetes during treatment with rosiglitazone.[20] The dose of rosiglitazone was selected on the basis that, of the regimens tested, this was associated with the greatest effect on plasma glucose in patients with type II diabetes,[21] and has a safety profile appropriate for investigative administration to patients with mild asthma.

Treatment with rosiglitazone was not associated with any effect on the EAR. The EAR is mediated by acute inflammatory mediators such as prostaglandins and leukotrienes; rosiglitazone was not expected to have an effect on this response. By contrast, treatment with rosigliatazone was associated with a statically significant reduction in the weighted mean LAR compared with placebo. The magnitude of the effect was however modest (15%). Clinically effective anti-inflammatories such as corticosteroids are associated with an approximately 90% attenuation of the LAR.[22,23] Montelukast, a leukotriene antagonist widely used in asthma but considered to have limited anti-inflammatory effects, has its principal effect on the EAR, but is also associated with approximately 25% attenuation of LAR.[23,24] In this context, the 15% attenuation associated with rosiglitazone is unlikely to be associated with a substantial impact during treatment of clinical asthma. The methacholine response on day 29 supported this conclusion: rosiglitazone required a lower concentration of methacholine to achieve at least a 20% fall in FEV_1 when compared with placebo, but this did not reach statistical or clinical significance. The dataset for the stand alone AMP challenge on day 14 is limited but is consistent with the response observed for the methacholine challenge.

Exhaled NO is widely used as a measure of airway inflammation.[25,26] Consistent with the LAR findings, treatment with rosiglitazone in this population of mild asthmatics was associated with a (pre-dose) reduction in FE_{NO} that was statistically greater than that observed during treatment with placebo. The blood biomarker data only show substantial effects on adiponectin, a marker of PPAR activation. Trends towards a reduction in inflammatory markers were observed (e.g. IL-4, IL-6, IL-13), but none of these were considered major effects. There were no trends observed in the blood differential white cell count.

Like all models, the allergen challenge has its limitations, but it is a sensitive measure of the potential for clinically effective anti-inflammatory drugs. All drugs that have clinically useful anti-inflammatory actions have a beneficial effect on the LAR.[27] In view of the modest effect on the allergen challenge the ability of the biomarker data to inform on the mechanism of the observed effect is limited. The observed reduction in FE_{NO} is consistent with *in vitro* observations that PPARγ agonist activity is associated with an inhibition of NO synthase. Although statistically significant the magnitude of the reduction is smaller than observed with inhaled corticosteroids (e.g. fluticasone).[28] The findings of this study are

consistent with *in vitro* studies but the concentrations associated with effects on nitric oxide synthase *in vitro* are observed at high concentrations (10 μM) and there is some evidence that these actions may be PPARγ independent.[29] This raises the possibility that much higher doses of a PPARγ agonist may be required to achieve all the anti-inflammatory effects observed in vitro; this is however somewhat speculative given the good evidence for substantial PPARγ activation seen in this study. In addition PPARγ agonists are associated with dose-related adverse effects such as weight gain (probably secondary to fluid retention), therefore substantially higher doses may not associated with a positive benefit/risk profile in asthma.

It is of note that Spears et al observed a clinically relevant improvement in FEV_1 in smoking asthmatics treated with rosiglitazone for 4 weeks. The population in that study is markedly more severe than that studied here (FEV 70—75% predicted vs 89% in this study; clinical requirement for inhaled corticosteroids).[5] Smoking asthmatics show corticosteroid insensitivity. The mechanisms underlying this are not fully characterized but have been postulated to involve a change in cytokine profile resulting in altered glucocorticoid receptor signalling, a reduction in the activity of HDAC, and an increase in competing molecular events.[30] Which of these may be responsible for the differences observed is not known.

Conclusion

Treatment with a PPARγ agonist (rosiglitazone) was associated with a modest (15%) reduction in the late asthmatic reaction in the allergen challenge model of asthma. This was accompanied by trends in other markers of efficacy and anti-inflammatory activity but none were considered major effects. In conclusion the findings of this study would imply that a PPARγ agonist alone is unlikely to be a clinically effective anti-inflammatory in mild asthma when used alone. The intriguing differences between the results of this study and another in smoking asthmatics would benefit from further mechanistic evaluation.

Conflict of interest statement

This study was funded by GlaxoSmithKline, UK. D. Richards, P. Bareille, E. Lindo, and S. Farrow are employees of GlaxoSmithKline (GSK), the manufacturer of AVANDIA. Dr Quinn is an employee of P3 research; P3 research was paid by GSK to conduct the study.

Author's contributions

DBR and SF designed the study. PB and DQ were responsible for conduct of the study. All authors were responsible for analysis and interpretation of the data, and for writing of the manuscript.

Acknowledgments

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Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.rmed.2009.11.006.

References

1. Martin G, Schoonjans K, Lefebvre AM, Staels B, Auwerx J. Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthetase genes by PPARalpha and PPARgamma activators. *J Biol Chem* 1997;**272**(45):28210—7.
2. Young PW, Cawthorne MA, Coyle PJ, Holder JC, Holman GD, Kozka IJ, et al. Repeat treatment of obese mice with BRL 49653, a new and potent insulin sensitizer, enhances insulin action in white adipocytes. Association with increased insulin binding and cell-surface GLUT 4 as measured by photoaffinity labeling. *Diabetes* 1995;**44**:1087—92.
3. Benayoun L, Letuve S, Druilhe A, Boczkowski J, Dombret MC, Mechighel P, Megret J, Leseche G, Aubier M, Pretolani M. Regulation of peroxisome proliferator-activated receptor gamma expression in human asthmatic airways: relationship with proliferation, apoptosis, and airway remodeling. *Am J Respir Crit Care Med* 2001;**164**(8 Pt 1):1487—94.
4. Belvisi MG, Hele DJ. Peroxisome proliferator-activated receptors as novel targets in lung disease. *Chest* 2008;**134**(1):152—7.
5. Spears M, Donnelly I, Jolly L, Brannigan M, Ito K, McSharry C, Lafferty J, Chaudhuri R, Braganza G, Bareille P, Sweeney L, Adcock IM, Barnes PJ, Wood S, Thomson NC. Bronchodilatory effect of the PPAR-gamma agonist rosiglitazone in smokers with asthma. *Clin Pharmacol Ther* 2009;**86**(1):49—53.
6. Ward JE, Tan X. Peroxisome proliferator activated receptor ligands as regulators of airway inflammation and remodelling in chronic lung disease. *PPAR Res* 2007;**2007**:14983.
7. Marx N, Kehrle B, Kohlhammer K, Grub M, Koenig W, Hombach V, Libby P, Plutzky J. PPAR activators as antiinflammatory mediators in human T lymphocytes: implications for atherosclerosis and transplantation-associated arteriosclerosis. *Circ Res* 2002;**90**(6):703—10.
8. Ueki S, Adachi T, Bourdeaux J, Oyamada H, Yamada Y, Hamada K, Kanda A, Kayaba H, Chihara J. Expression of PPARgamma in eosinophils and its functional role in survival and chemotaxis. *Immunol Lett* 2003;**86**(2):183—9.
9. Mueller C, Weaver V, Vanden Heuvel JP, August A, Cantorna MT. Peroxisome proliferator-activated receptor gamma ligands attenuate immunological symptoms of experimental allergic asthma. *Arch Biochem Biophys* 2003;**418**(2):186—96.
10. Woerly G, Honda K, Loyens M, Papin JP, Auwerx J, Staels B, Capron M, Dombrowicz D. Peroxisome proliferator-activated receptors alpha and gamma down-regulate allergic inflammation and eosinophil activation. *J Exp Med* 2003;**198**(3):411—21.
11. Trifilieff A, Bench A, Hanley M, Bayley D, Campbell E, Whittaker P. PPAR-alpha and -gamma but not -delta agonists inhibit airway inflammation in a murine model of asthma: in vitro evidence for an NF-kappaB-independent effect. *Br J Pharmacol* 2003;**139**(1):163—71.
12. Hashimoto Y, Nakahara K. Improvement of asthma after administration of pioglitazone. *Diabetes Care* 2002;**25**(2):401.

13. Young PW, Buckle DR, Cantello BCC, Chapman H, Clapham JC, Coyle PJ, et al. Identification of high-affinity binding sites for the insulin-sensitizer rosiglitazone (BRL 49653) in rodent and human adipocytes using a radioiodinated ligand for peroxisomal proliferator-activated receptor gamma. *J Pharmacol Exp Ther* 1998;**284**:751—9.
14. Seimandi M, Lemaire G, Pillon A, Perrin A, Carlavan I, Voegel JJ, Vignon F, Nicolas JC, Balaguer P. Differential responses of PPARalpha, PPARdelta, and PPARgamma reporter cell lines to selective PPAR synthetic ligands. *Anal Biochem* 2005;**344**(1):8—15.
15. Silvestri M, Oddera S, Sacco O, Balbo A, Crimi E, Rossi GA. Bronchial and bronchoalveolar inflammation in single early and dual responders after allergen inhalation challenge. *Lungs* 1997;**175**(4):277—85.
16. Grzelewska-Rzymowska I, Gondorowicz K, Cieslewicz G, Roznieckl J. Course of non-specific bronchial reactivity to histamine after bronchospasm induced by allergen challenge in patients with bronchial asthma. *Pneumonol Alergol Pol* 1995; **63**(5—6):273—80.
17. Joos GF, O'Connor B, Anderson SD, Chung F, Cockcroft DW, Dahlen B, DiMaria G, Foresi A, Hargreave FE, Holgate ST, Inman M, Lotvall J, Magnussen H, Polosa R, Postma DS, Riedler J. ERS Task Force. Indirect airway challenges. *Eur Respir J* 2003;**21**(6):1050—68.
18. van den Berge M, Kerstjens HA, Meijer RJ, de Reus DM, Koeter GH, Kauffman HF, Postma DS. Corticosteroid-induced improvement in the PC20 of adenosine monophosphate is more closely associated with reduction in airway inflammation than improvement in the PC20 of methacholine. *Am J Respir Crit Care Med* 2001;**164**(7):1127—32.
19. Mohanty P, Aljada A, Ghanim H, Hofmeyer D, Tripathy D, Syed T, Al-Haddad W, Dhindsa S, Dandona P. Evidence for a potent antiinflammatory effect of rosiglitazone. *J Clin Endocrinol Metab* 2004;**89**(6):2728—35.
20. Yang WS, Jeng CY, Wu TJ, Tanaka S, Funahashi T, Matsuzawa Y, Wang JP, Chen CL, Tai TY, Chuang LM. Synthetic peroxisome proliferator-activated receptor-gamma agonist, rosiglitazone, increases plasma levels of adiponectin in type 2 diabetic patients. *Diabetes Care* 2002;**25**(2):376—80.
21. Phillips LS, Grunberger G, Miller E, Patwardhan R, Rappaport EB, Salzman A. Rosiglitazone Clinical Trials Study Group. Once- and twice-daily dosing with rosiglitazone improves glycemic control in patients with type 2 diabetes. *Diabetes Care* 2001;**24**(2):308—15.
22. Kidney JC, Boulet LP, Hargreave FE, et al. Evaluation of single-dose inhaled corticosteroid activity with an allergen challenge model. *J Allergy Clin Immunol* 1997;**100**:65—70.
23. Palmqvist M, Bruce C, Sjostrand M, Arvidsson P, Lotvall J. Differential effects of fluticasone and montelukast on allergen-induced asthma. *Allergy* 2005;**60**:65—70.
24. Singh D, Richards D, Knowles RG, Schwartz S, Woodcock A, Langley S, O'Connor BJ. Selective inducible nitric oxide synthase inhibition has no effect on allergen challenge in asthma. *Am J Respir Crit Care Med* 2007;**176**(10):988—93.
25. Alving K, Weitzberg E, Lundberg JM. Increased amount of nitric oxide in exhaled air of asthmatics. *Eur Respir J* 1993;**6**: 1368—70.
26. Kharitonov SA, Yates D, Robbins RA, et al. Increased nitric oxide in exhaled air of asthmatic patients. *Lancet* 1994;**343**: 133—5.
27. Hansel TT, Erin EM, Barnes PJ. The allergen challenge. *Clin Exp Allergy* 2002;**32**(2):162—7.
28. van Rensen EL, Straathof KC, Veselic-Charvat MA, Zwinderman AH, Bel EH, Sterk PJ. Effect of inhaled steroids on airway hyperresponsiveness, sputum eosinophils, and exhaled nitric oxide levels in patients with asthma. *Thorax* 1999 May; **54**(5):403—8.
29. Crosby MB, Svenson JL, Zhang J, Nicol CJ, Gonzalez FJ, Gilkeson GS. Peroxisome proliferation-activated receptor (PPAR)gamma is not necessary for synthetic PPARgamma agonist inhibition of inducible nitric-oxide synthase and nitric oxide. *J Pharmacol Exp Ther* 2005;**312**(1):69—76.
30. Thomson NC, Shepherd M, Spears M, Chaudhuri R. Corticosteroid insensitivity in smokers with asthma: clinical evidence, mechanisms, and management. *Treat Respir Med* 2006;**5**(6):467—81.